UNIVERSITI PUTRA MALAYSIA

REVERSE TRANSCRIPTASE IN SITU EXPRESSION PATTERNS OF P53, CYCLIN E AND RB GENES AT DIFFERENT STAGES OF BREAST CANCER

MOHAMMADREZA ZAMANIAN

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By

MOHAMMADREZA ZAMANIAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Masters of Science

May 2007

In dedication to:

My beloved wife Zeinab, who has supported me in all of my life events, particularly in raising the decision to change our future

And

To my son Alisina, for giving soul to our life

I hope I can make up the lost time with you

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in partial fulfilment of the requirement for the degree of Master of Science

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May 2007

Chairperson: **Associate Professor Patimah Ismail, PhD**

Faculty: **Medicine and Health Sciences**

Breast cancer is one of the most important health problems among females. One of the most important challenges regarding breast cancer diagnosis and treatment is the precise clinical staging of the disease. With regards to the selection of appropriate treatment method, identifying the lymph node involvement by cancerous cells is a major determinant. Until now, many attempts have been made to find stage specific molecular markers to help the clinicians make precise staging of the disease. Through this, evaluating the activity of some important genes in cancer evolution and progression seems to be the most sensible step in this direction.

p53, Cyclin E and Rb are genes that are mostly interactive in cell cycle regulation and cell division as well, has been shown to have an important role in cancer development particularly in breast cancer. Abnormalities in their inhibitory and or stimulatory roles in cell cycle progression can lead cells to enter hyperproliferative or neoplastic states. Therefore, assessments to determine their activity can lead to finding any differences in their expression levels between benign and malignant breast tissues, as well as different stages of breast cancer.

In the present study we have used Reversed Transcriptase in situ Polymerase Chain Reaction (RT *in situ* PCR) in order to determine p53, Cyclin E and Rb mRNA expressions in different human breast samples including benign and malignant tissues. This method allows detection of very low copies of mRNA at cellular level.

In the current study, the presence of p53, Cyclin E and Rb mRNA expressions were investigated in 17 cases of human breast tissues, which were donated as paraffin embedded materials by the pathology ward of Milad hospital, located in Tehran, Iran.

We divided the samples into four groups based on their pathology reports. Five samples in each first group as named; non-malignant human breast lesions or NM, lymph node negative human breast cancer (No regional lymph node involvement; LNN) and lymph node positive human breast cancer (Positive for regional lymph node involvement; LNP). There were just two samples available in the fourth group of our study as extra nodal metastatic human breast cancer (Positive for distant metastasis; MB).

Our data analysis was mostly based on qualitative assessment of the images which includes the presence of expression in tissue sections as well as the location of the signals throughout the tissue and inside the cells. In addition, we did statistical analysis to compare the abundance of expression among different categories of our samples. Analysis of the data showed that the closest results to significant level (<0.05) were those comparing benign and malignant groups especially for Rb mRNA. While, the most improbable results to significant level were those comparing among four study groups especially between LNP and MB.

Our findings demonstrated a dominant presence of p53 and Cyclin E mRNA expression in malignant breast tissues as compared to benign lesions. On the contrary, benign breast lesions showed a more dominant expression of Rb mRNA than malignant tissues.

A comparison between different breast cancer groups in our study showed slight differences in the proportions and intensities of p53, Cyclin E and RB mRNA expressions. These differences could be meaningful but the nature of our study, which was a qualitative method of research, does not allow definitive inference from the findings.

In conclusion, RT *in situ* PCR as a qualitative method is able to localize mRNA gene expression in human breast lesions. In addition, mRNA expression levels are obviously different in benign tissues compared to malignant tissues. How ever, it is not possible to rely on the slight differences between three malignant groups of our study. It is therefore necessary to do further investigations with quantitative research methods such as microarray analysis and or quantitative RT- PCR.

Abstrak tesis yang dikemuleakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PATEN EKSPRESI RT IN SITU PCR DALAM P53,CYCLIN E DAN RB GEN DALAM TAHAP KANSER PAYUDARA YANG BERLAINAN

Oleh

MOHAMMADREZA ZAMANIAN

May 2007

Pengerusi : Profesor Madya Patimah Ismail, PhD

Fakulti : Perubatan dan Sains Kesihatan

Kanser payudara adalah masalah kesihatan yang paling utama yang dihidapi oleh golongan wanita. Salah satu cabaran utama dalam mendiagnosa dan pengubatan kanser payudara adalah dalam kepersisan dalam mengkelasifikasikan peringkat-peringkat kanser. Dalam pemilihan cara pengubatan yang paling bersesuaian, pengenalpastian kalenjar limfa yang terlibat juga merupakan salah satu cara yang utama. Sehingga sekarang, pelbagai cubaan telah dijalankan untuk mencari marker molecular spesifik dengan tujuan untuk membantu para pakar surgen dan Perubatan kedal dan Qulcologia untuk membuat keputusan tepat mengenai peringkat-peringkat kanser payudara. Melaluinya, penilaian aktiviti sesetengah gen penting di dalam evolusi dan perkembangan kanser didapati penting didalam pemilihan jenis rawatan kanser berkenaan.

p53, Cyclin E dan Rb adalah gen yang terlibat dalam kitaran dan pembahagian sel, telah dibukti memainkan peranan penting dalam pembentukan kanser, terutamanya kanser payudara. Ketidaknormalan peranan gen tersebut di dalam proses menyekat dan/atau mencetus perkembangan kitaran sel boleh menyebabkan sel memasuki peringkat hiperpoliferasi malahan neoplastik. Oleh itu, penilaian aktiviti gen tersebut

boleh membawa kepada penemuan sebarang perbezaan tahap ekpresi di antara benigna dan malignan, termasuk peringkat-peringkat kanser payudara.

Di dalam kajian ini, kami telah menggunakan Reversed Transcriptase *in situ* Polymerase Chain Reaction (RT *in situ* PCR) untuk menentukan ekpresi p53, Cyclin E dan Rb mRNA dalam sampel payudara manusia yang berbeza termasuklah tisu benigna dan malignan. Kaedah ini membolehkan jumlah mRNA yang sangat rendah pada peringkat sel dikenalpasti.

Kami telah mengkaji kehadiran ekpresi p53, Cyclin E dan Rb mRNA dalam 17 kes tisu payudara manusia yang tertanam dalam "paraffin" yang didermakan oleh wad patologi Hospital Milad yang terletak di Tehran, Iran.

Kami telah membahagikan sampel kepada empat kumpulan berdasarkan laporan patologi. Lima sampel di dalam setiap kumpulan; lesi keabnormalan payudara manusia benigna, kanser payudara manusia negatif nodus limfa dan kanser payudara manusia positif nodus limfa. Hanya terdapat dua sampel dalam kumpulan keempat kajian kami sebagai kanser payudara manusia metastatik.

Perbandingan di antara kumpulan-kumpulan kanser payudara dalam kajian kami menunjukan sedikit perbezaan nisbah dan kekuatan ekpresi p53, Cyclin E dan Rb mRNA. Walaupun perbezaan ini boleh digunapakai, tetapi adalah berkemungkinan ianya tidak tepat kerana kami menggunakan kaedah kualitatif dan sampel yang terhad.

Kesimpulannya, RT *in situ* PCR adalah kaedah kualitatif yang boleh mengenalpasti lokasi ekpresi gen mRNA dalam tisu payudara manusia. Tambahan pula, tahap ekpresi mRNA adalah nyata berbeza di antara tisu benign berbanding dengan tisu malignan. Bagi membolehkan perbezaan yang sedikit di antara tiga kumpulan malignan dalam kajian kami diambil kira, adalah perlu untuk melakukan kajian yang lebih mendalam termasuk kaedah kajian kuantitatif seperti analisis microarray dan/atau quantitative RT-PCR.

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I certify that an Examination Committee has met on 25th May 2007 to conduct the final examination of Mohammadreza Zamanian on his Master of Science thesis entitled "Reverse Transcriptase *In situ* Expression Patterns of p53, Cycline and Rb Genes at Different Stages of Breast Cancer" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Masters of Science. The members of the Supervisory Committee are as follows:

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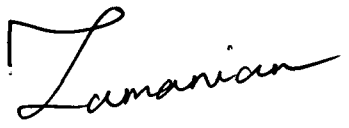
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



MOHAMMADREZA ZAMANIAN

Date: 2nd August 2007

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LIST OF ABBREVIATIONS

AMV	Alfa Mosaic Virus
bp	Base pair
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
DAB	3,3' -diaminobenzidine
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
DPX	DePex
dATP	Deoxyadenosine triphosphate
dNTP	Deoxy nucleic triphosphate
dUTP	Deoxy-uridine-5' triphosphate
EDTA	Ethylene diamine tertra acetate
et al.	*et alii*
ETOH	Ethanol
H&E	Hematoxylin and Eosin
LNN	Lymph node negative (No lymph node involvement)
LNP	Lymph node positive (Presence of lymph node metastasis)
MAL	Malignant
MB	Metastatic breast tissue (Presence of distant metastasis)
mRNA	Messenger ribonucleic acid
NaCl	Sodium chloride
NM	Non-malignant
PBS	Phosphate buffered saline
RNA	Ribonucleic acid
rt	Room temperature

RT-PCR	Reverse transcriptase-polymerase chain reaction
TBE	Tris-boric acid disodium EDTA
TE	Tris EDTA
Tfl	*Thermus flavus*
Tris	Tris(hydromethyl) aminomethane

CHAPTER 1

INTRODUCTION

Breast cancer is a great problem of human's and especially women's health at the present time. It is diagnosed one million times every year worldwide (Berns *et al.* 2004). It is the second leading cause of cancer deaths in women today (after lung cancer) and the most common cancer among women, excluding nonmelanoma skin cancers. In Iran, breast cancer continues to increase in numbers yearly and remains an important health problem, although its statistics is very similar to that of other countries in the region (Harirchi *et al.* 2002).

One of the most important issues in improving health indices regarding breast cancer is using individualized treatment methods. Strategies for treatment in breast cancer depends on the extent of disease progression in the body that will be evaluated by certain criteria; size of the tumor mass, lymph node involvement and the presence of metastasis which is based on TNM system (T; tumor size, N; lymph node involvement and M; metastasis). In order to help the clinicians to decide on treatment modalities, it is necessary to have a standard method for determining disease progression. Breast cancer is usually divided into four stages based on above criteria. There are different prognoses, choices of treatment, response rates to therapy and survival in each of different stages of breast cancer. In order to use targeted therapy clinicians need to know the precise stage of a breast cancer, which determines the extent of disease